Applied and Environmental Microbiology, Feb. 2011, p. 1127–1131 Vol. 77, No. 3
0099-2240/11/$12.00 doi:10.1128/AEM.02130-10

Effect of a New Probiotic *Saccharomyces cerevisiae* Strain on Survival of *Escherichia coli* O157:H7 in a Dynamic Gastrointestinal Model[▽]

Lucie Etienne-Mesmin,[1,2] Valérie Livrelli,[2] Maud Privat,[2] Sylvain Denis,[1] Jean-Michel Cardot,[1] Monique Alric,[1] and Stéphanie Blanquet-Diot[1]*

Clermont Université, Université d'Auvergne, Centre de Recherche en Nutrition Humaine Auvergne, ERT 18, Conception Ingénierie et Développement de l'Aliment et du Médicament, BP 10448, F-63000 Clermont-Ferrand, France,[1] and Clermont Université, Université d'Auvergne, Centre de Recherche en Nutrition Humaine Auvergne JE 2526 USC INRA 2018, Evolution des Bactéries Pathogènes et Susceptibilité Génétique de l'Hôte, BP 10448, F-63000 Clermont-Ferrand, France[2]

Received 9 September 2010/Accepted 22 November 2010

Survival of *Escherichia coli* O157:H7 was investigated using a dynamic gastrointestinal model. A high bacterial mortality was observed in the stomach and duodenum. In contrast, bacteria grew in the distal parts of the small intestine. The coadministration of *Saccharomyces cerevisiae* CNCM I-3856 led to a significant reduction of bacterial resumption, maybe through ethanol production.

Enterohemorrhagic *Escherichia coli* (EHEC) is a food-borne pathogen that causes human diseases ranging from uncomplicated diarrhea to hemorrhagic colitis and hemolytic-uremic syndrome. Human contamination occurs mainly by ingestion of raw ground beef, vegetables, water, and dairy products contaminated by cattle feces (3, 25). Most of the outbreaks and sporadic cases of infection around the world are caused by EHEC strains that belong to serotype O157:H7 (24, 26). The virulence of EHEC strains is associated with the ability of the bacteria to attach to and efface intestinal brush borders and produce Shiga toxins Stx1 and/or Stx2. The terminal ileum and the colon are supposed to be the principal sites of EHEC colonization and pathology in humans (10, 15, 24).

To cause human illness, *E. coli* O157:H7 must survive the gastric and small intestinal environment in transit toward the large intestine. Gastric pH, gastrointestinal secretions, and peristalsis are well-known factors controlling the outcome of food-borne pathogens. However, few reports have really focused on the behavior of *E. coli* O157:H7 in the human gastrointestinal tract. The available data (1, 4, 27, 30) were obtained in gastric static systems that are not representative of the continuously changing variables during chyme transit. The TNO gastrointestinal tract model (TIM; Zeist, Netherlands) is an alternative dynamic multicompartmental *in vitro* system which presently allows the closest simulation of *in vivo* physiological processes occurring within the lumen of the stomach and small intestine of human (22). It consists of four successive compartments simulating the stomach, duodenum, jejunum, and ileum of human. The main parameters of digestion, such as pH, body temperature, peristaltic mixing and transport, gastric, biliary, and pancreatic secretions, and passive absorption of small molecules and water, are reproduced as accurately as possible. One of the main advantages of the TIM system is the possibility to collect digestive samples at any level of the gastrointestinal tract and at any time during digestion. This model has been validated for microbial applications and is particularly relevant for probiotics (6, 21) and pathogenic microorganism (18) survival studies.

Since antibiotic therapy is regarded as controversial in the treatment of EHEC infections (33), probiotics are being investigated as an alternative strategy. Probiotics are defined as live microorganisms that resist digestion and reach the colon alive and, when administered in adequate amounts, confer a health benefit on the host (14). To control EHEC infection, probiotic administration may be considered at the following two levels: (i) in ruminants to decrease *E. coli* O157:H7 carriage and therefore reduce the risk of human food-borne disease or (ii) in humans to exert a direct antagonist effect against the pathogen. Probiotic bacteria or yeasts have been evaluated for their capacity to reduce EHEC growth in batch cultures (2, 23), decrease the infectious proinflammatory response in infected T84 cells (12), or limit EHEC fecal shedding in cattle (32).

Until now, *Saccharomyces cerevisiae* var. *boulardii* was the only yeast commercialized as a probiotic for human use. *S. cerevisiae* CNCM I-3856 is a new marketed yeast probiotic (Lynside Pro GI+, Lesaffre Human Care, Milwaukee, WI) which has already shown anti-inflammatory activities in induced colitis in mice (16) and efficiency in reducing digestive discomfort and abdominal pain in patients with irritable bowel syndrome (IBS) (13).

In this work, we describe the use of the TIM system to evaluate the survival of an *E. coli* O157:H7 strain in simulated human gastrointestinal conditions and the influence of *S. cerevisiae* CNCM I-3856 on bacterial viability. To closely mimic physiological conditions, the pathogen (with or without yeast) was given in a typical Western diet containing raw ground beef, one of the main foods involved in *E. coli* O157:H7 outbreaks.

Artificial digestions in the TIM system. An aerobic culture (LB, 24 h, 37°C) of an isogenic mutant of *E. coli* O157:H7

* Corresponding author. Mailing address: Equipe de Recherche Technologique Conception, Ingénierie et Développement de l'Aliment et du Médicament (ERT CIDAM), Université Clermont-Ferrand I, Faculté de Pharmacie, 28 place Henri-Dunant, 63001 Clermont-Ferrand, France. Phone: (33) 473 17 83 90. Fax: (33) 473 17 83 92. E-mail: stephanie.blanquet@u-clermont1.fr.

▽ Published ahead of print on 3 December 2010.

TABLE 1. Parameters of gastrointestinal digestion in the TIM system when simulating digestive conditions of a healthy adult after intake of a solid meal[a]

Compartment	Vol (ml) at initial time	pH/time (min)	Secretion	$t_{1/2}$ (min)	ß coefficient
Gastric	300	2/0, 6/5, 5.7/15, 4.5/45, 2.9/90, 2.3/120, 1.8/240, 1.6/300	0.25 ml/min of pepsin (2,080 IU/ml), 0.25 ml/min of lipase (250.5 IU/ml), 0.25 ml/min of HCl (1.5 M) if necessary	85	1.8
Duodenal	30	Maintained at 6.0	0.5 ml/min of bile salts (4% during the first 30 min of digestion and then 2%), 0.25 ml/min of pancreatic juice (10^3 USP/ml), 0.25 ml/min of intestinal electrolyte solution, 0.25 ml/min of $NaHCO_3$ (1 M) if necessary, 23,600 IU of trypsin (at the beginning of digestion)		
Jejunal	130	Maintained at 6.9	0.25 ml/min of $NaHCO_3$ (1 M) if necessary		
Ileal	130	Maintained at 7.2	0.25 ml/min of $NaHCO_3$ (1 M) if necessary	250	2.5

[a] The power exponential equation ($f = 1 - 2^{-(t/t_{1/2})\beta}$, where f represents the fraction of meal delivered, t the time of delivery, $t_{1/2}$ the half-time of delivery, and β a coefficient describing the shape of the curve) is used for the computer control of gastric and ileal deliveries in the TIM.

strain EDL 933 lacking the stx_1 and stx_2 genes (19) was used to inoculate the test meal (2.5×10^5 CFU/ml) prior to its introduction into the TIM system. This mutant has been widely used to study EHEC infectious processes (10, 20, 28). When coadministrated with bacteria, *S. cerevisiae* CNCM I-3856 was given in its active dried powder form (10^7 CFU/ml). The TIM system was programmed to reproduce the digestion of a solid meal in a healthy human adult (Table 1). The total duration of the digestions was 300 min ($n = 4$ digestions for each condition). Samples were taken in the test meal (initial intake) before its introduction into the artificial stomach and regularly collected during digestion in the different com-

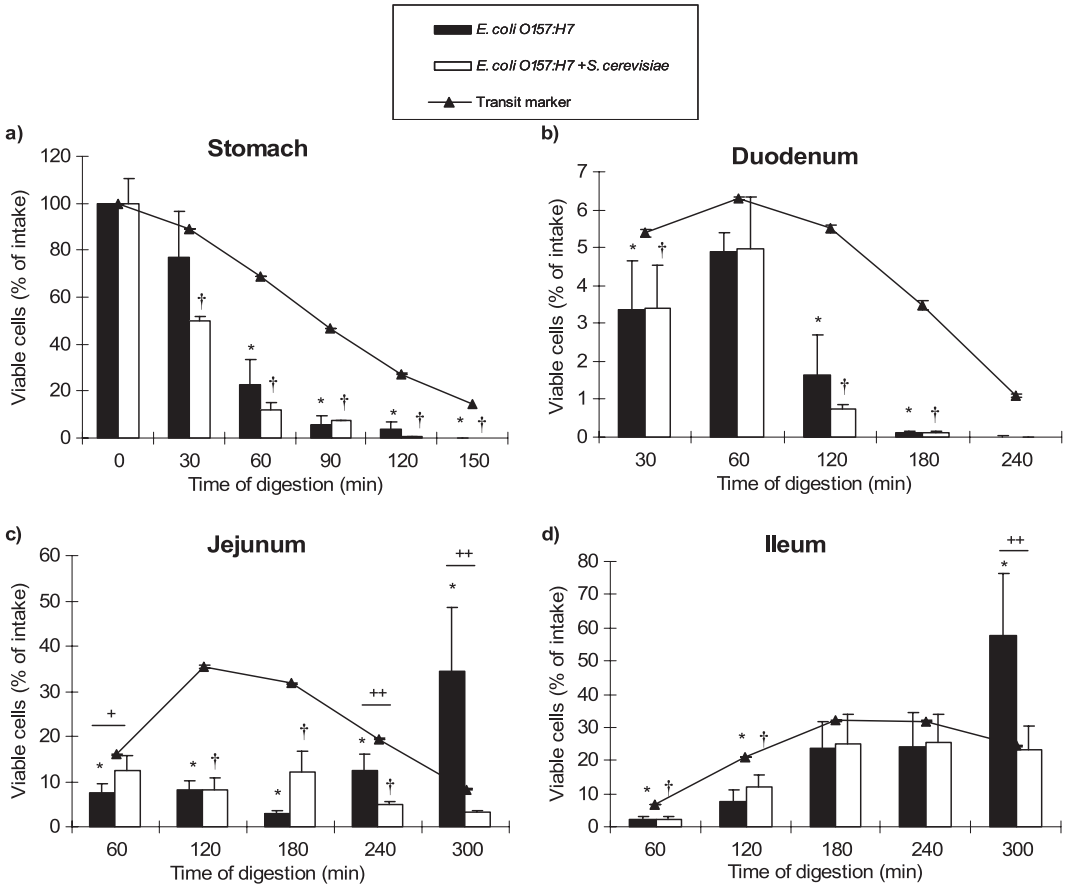

FIG. 1. Influence of *S. cerevisiae* CNCM I-3856 on the survival of *E. coli* O157:H7 during *in vitro* digestions in the stomach (a), duodenum (b), jejunum (c), and ileum (d) of the TIM. The results obtained in each compartment for bacteria without (black bar) or with (white bar) yeast are compared with that of the transit marker, blue dextran (black line). Results are expressed as mean percentages ± standard deviations (SD) ($n = 4$ digestions) of initial intake. *E. coli* O157:H7 (*) and *E. coli* O157:H7 + *S. cerevisiae* (†) results significantly different from those of the transit marker ($P < 0.05$). *E. coli* O157:H7 results significantly different from those of *E. coli* O157:H7 plus *S. cerevisiae* at P values of <0.05 (+) or P values of <0.01 (++).

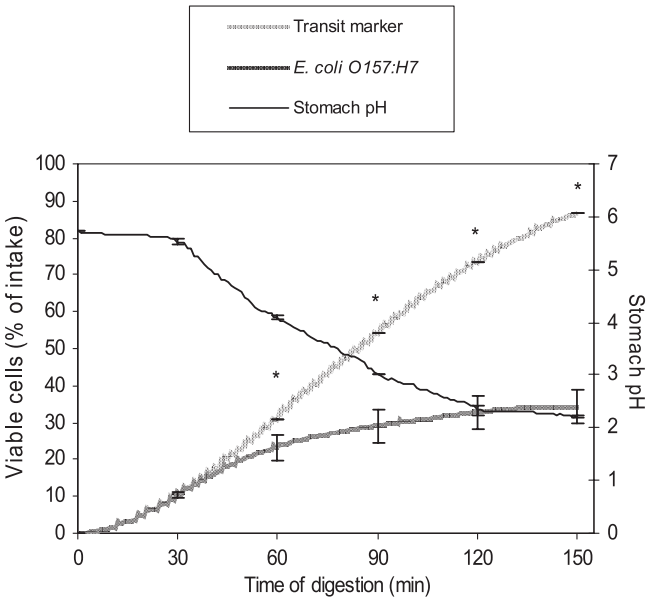

FIG. 2. Cumulative gastric delivery of viable *E. coli* O157:H7 (thick black line) and blue dextran (thick gray line) in the TIM. Results are expressed as mean percentages ± SD ($n = 4$ digestions) of initial intake. The fall of gastric pH during *in vitro* digestions is plotted (thin black line). *, *E. coli* O157:H7 results significantly different from those of the transit marker ($P < 0.05$).

partments of the system (stomach, duodenum, jejunum, and ileum). Microbial counting was performed on sorbitol-MacConkey agar (bacteria) and on Sabouraud dextrose agar (yeast). To evaluate bacterial and yeast survival rates in the TIM, control digestions ($n = 4$) were carried out in the same experimental conditions with water containing 0.8% (wt/vol) of a nonabsorbable transit marker, blue dextran (22). The concentrations of ethanol in the digestive samples were determined using an enzymatic UV test (Biosentec, Toulouse, France). Significant differences between treatments were tested by analysis of variance (ANOVA) with repeated-measure analysis followed by a post hoc test (SAS 9.1 Software, Inc., Cary, NC). *P* values of <0.05 were taken to indicate statistical differences.

Survival of *E. coli* O157:H7 in simulated gastrointestinal conditions. The viability of *E. coli* O157:H7 was evaluated in each TIM compartment by comparing the curves obtained for the bacteria and for the transit marker (Fig. 1). Our results showed that during its transit through the stomach (Fig. 1a) and duodenum (Fig. 1b) of the TIM, *E. coli* O157:H7 was largely killed, probably due to the occurrence of stringent conditions such as gastric acidity, digestive enzymes, and bile salts. In particular, bile salts are toxic at high concentrations for bacterial cells by disorganizing the lipid bi-layer structure of the cellular membranes (31). At the end of gastric digestion, 34% ± 4% of the initial bacteria are still alive and are in transit to the small intestine (Fig. 2). Even if acid resistance features have been described for *E. coli* O157:H7 strains (17), these bacteria were found to be sensitive to low pH in the present study. Indeed, cell mortality was observed in the artificial stomach from 60 min, when the pH fell below 4 (Fig. 2). Large variations in survival rates (from 0 to 100%) were observed for *E. coli* O157:H7 in *in vitro* gastric models (1, 4, 27, 29, 30). This wide range of values may be explained by differences between culture conditions, digestive systems (static or dynamic) and parameters, food matrices, and also bacterial strains. Interestingly, the only study that evaluated the behavior of *E. coli* O157:H7 in dynamic conditions (reproduction of the fall in gastric pH) gave survival percentages after gastric digestion close to those obtained in our study (29).

In the distal parts of the artificial gastrointestinal tract, growth resumption was observed at the end of digestion. At 300 min, the percentages for bacteria largely exceeded that of the transit marker in the jejunum (35% ± 14% versus 8% ± 1%) (Fig. 1c) and in the ileum (58% ± 19% versus 25% ± 1%) (Fig. 1d). This growth resumption was probably linked to less stringent environmental conditions, such as a pH closer to neutrality, lower concentrations of bile salts (owing to their passive reabsorption), and/or an increase in the residence time of bacteria. This study is the first report on the behavior of *E. coli* O157:H7 in human small-intestinal conditions. Bacterial growth renewal has been already observed in the small-intestinal compartments of the TIM but for a nonpathogenic strain of *E. coli* (18).

Antagonistic effect of *S. cerevisiae* CNCM I-3856. The lack of specific treatment for EHEC infections has prompted work on alternative preventive and/or curative strategies, such as the use of probiotics. Among potential candidates, we evaluated the influence of a new probiotic *S. cerevisiae* strain on the viability of *E. coli* O157:H7 in the TIM system. The survival of *E. coli* O157:H7 in the stomach and duodenum of the artificial digestive system was not significantly modified by the coadministration of *S. cerevisiae* CNCM I-3856 (Fig. 1a and b). On the contrary, at the end of digestion (300 min), the addition of yeast led to a 10-fold decrease in bacterial survival in the jejunum ($P = 0.0008$) (Fig. 1c). Similarly, 58% ± 19% of the bacteria initially introduced in the TIM was recovered after 300 min of digestion in the ileum when bacteria were administered alone, whereas only 23% ± 7% of bacteria was found when yeasts were added in the test meal ($P = 0.0057$) (Fig. 1d). For the first time, we highlighted a direct antagonistic effect of *S. cerevisiae* against an EHEC strain in simulated human conditions. This interesting result suggests that this yeast could be used in humans to limit the amount of *E. coli* O157:H7 that reaches the distal small intestine and the colon. The antagonistic effect observed *in vitro* may be explained by (i) the competition between yeasts and bacteria for nutrient utilization, (ii) the modification by yeasts of the physico-chemical conditions of the digestive environment (i.e., redox potential), or (iii) the production by yeasts of inhibitory substances (11), such as proteases (7, 8) or ethanol.

To further investigate the antagonistic mechanisms between bacteria and yeast, ethanol concentrations were measured in the intestinal compartments of the TIM. While near-zero levels were observed in samples collected during digestions with *E. coli* O157:H7 alone, the ethanol contents reached 0.58 ± 0.15 g/liter when *S. cerevisiae* CNCM I-3856 was added in the test meal ($P < 0.01$) (Fig. 3). We therefore showed, for the first time, that an *S. cerevisiae* strain is able to produce ethanol directly in the human digestive environment. Ethanol, like other organic solvents, may be lethal for bacteria by disrupting

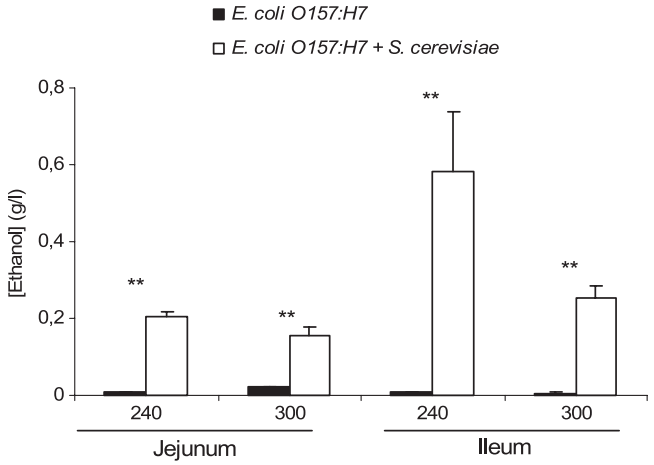

FIG. 3. Ethanol concentrations in jejunal and ileal samples from the TIM. **, *E. coli* O157:H7 results significantly different from those of *E. coli* O157:H7 plus *S. cerevisiae* ($P < 0.01$).

the membrane to repress cell growth (9). Since yeasts synthesize ethanol from simple sugars released during digestion, it was not surprising to observe high levels of ethanol in the distal parts of the small intestine.

Survival of *S. cerevisiae* CNCM I-3856 in simulated gastrointestinal conditions. Our results also showed a high resistance of *S. cerevisiae* CNCM I-3856 to gastric and small-intestinal secretions and low gastric pH (Fig. 4). This result may encourage the use of this yeast in comparison to lactic acid bacteria in a probiotic strategy. Indeed, survival rates for *Lactobacillus* spp. and *Streptococcus* spp. during digestion in the TIM were much lower than those obtained in this study for yeasts (6, 21). In addition, our previous works on other *S. cerevisiae* strains showed the same yeast viability and also indicated that the survival rates obtained *in vitro* in TIM systems are consistent with *in vivo* data in humans (5).

In conclusion, our *in vitro* experiments provide new information on the survival of *E. coli* O157:H7 in the overall upper gastrointestinal tract of humans. Such data are essential for a full understanding of EHEC pathogenesis and for setting regulatory standards in the food processing industry. The *S. cerevisiae* CNCM I-3856 yeast strain appears to exert antagonistic effects against this enteric pathogen in the distal part of the small intestine, maybe through ethanol production. This property should be exploited for the development of prophylactic and/or therapeutic agents involved in the control of EHEC infections. The mode of action of *S. cerevisiae* CNCM I-3856, and particularly its interaction with the human intestinal mi-

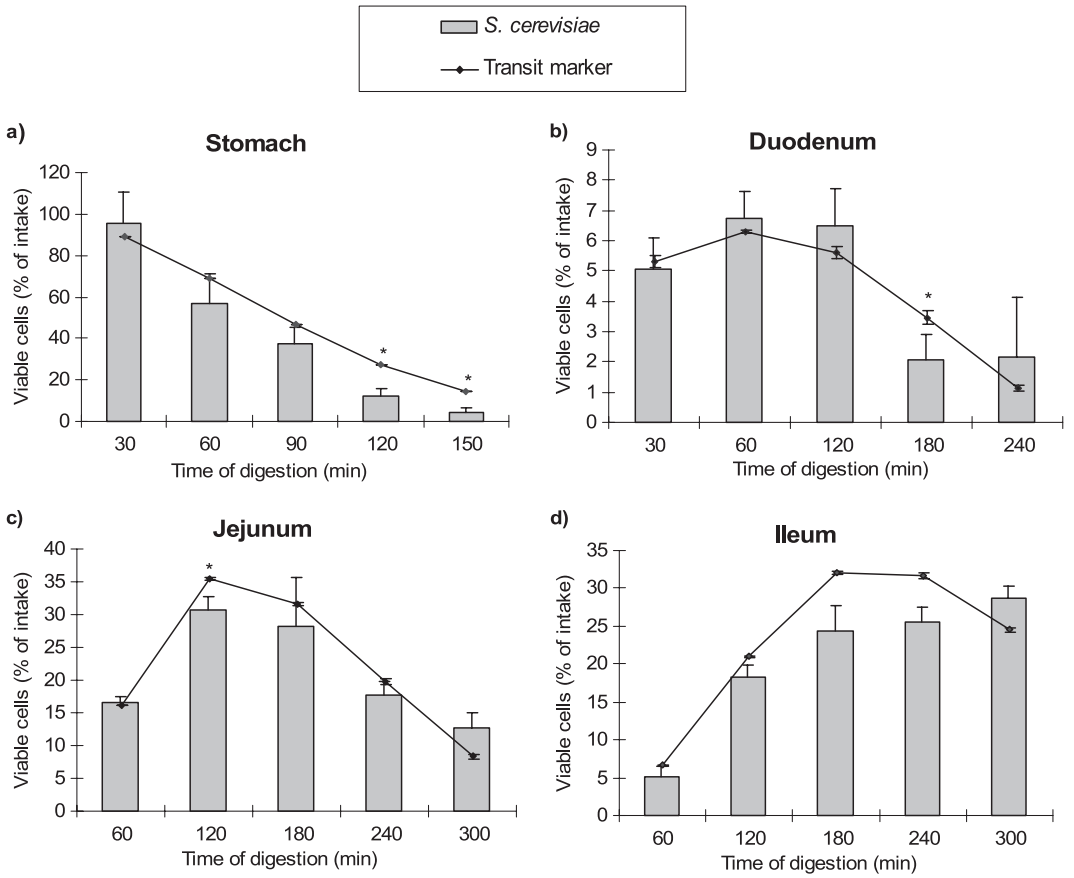

FIG. 4. Survival of *S. cerevisiae* CNCM I-3856 during *in vitro* digestions in the stomach (a), duodenum (b), jejunum (c), and ileum (d) of the TIM. The results obtained in each compartment for yeasts (gray bars) are compared with that of the transit marker, blue dextran (black line). Results are expressed as mean percentages ± SD ($n = 4$ digestions) of initial intake. *, *S. cerevisiae* results significantly different from those of the transit marker ($P < 0.05$).

crobiota, now needs to be further investigated using *in vitro* and *in vivo* approaches.

This work was supported by grants from the French Ministère de l'Education Nationale, de l'Enseignement Supérieur et de la Recherche to L.E.-M., ERT-CIDAM, and JE2526 USC-INRA 2018.

We thank Christine Martin (Unité de Microbiologie, INRA Clermont-Ferrand Theix) for the generous gift of the *E. coli* O157:H7 mutant. We also thank Lesaffre Company for providing the *S. cerevisiae* CNCM I-3856 strain. We are grateful to Sandrine Chalancon for technical assistance during the experiments on the TIM system.

REFERENCES

1. **Arnold, K. W., and C. W. Kaspar.** 1995. Starvation- and stationary-phase-induced acid tolerance in *Escherichia coli* O157:H7. Appl. Environ. Microbiol. **61:**2037–2039.
2. **Bach, S. J., T. A. McAllister, D. M. Veira, V. P. J. Gannon, and R. A. Holley.** 2003. Effects of a *Saccharomyces cerevisiae* feed supplement on *Escherichia coli* O157:H7 in ruminal fluid in vitro. Anim. Feed Sci. Technol. **104:**179–189.
3. **Berger, C. N., et al.** 2010. Fresh fruit and vegetables as vehicle for the transmission of human pathogens. Environ. Microbiol. **12:**2385–2397.
4. **Bergholz, T. M., and T. S. Whittam.** 2007. Variation in acid resistance among enterohaemorrhagic *Escherichia coli* in a simulated gastric environment. J. Appl. Microbiol. **102:**352–362.
5. **Blanquet, S., J. P. Meunier, M. Minekus, S. Marol-Bonnin, and M. Alric.** 2003. Recombinant *Saccharomyces cerevisiae* expressing P450 in artificial digestive systems: a model for biodetoxication in the human digestive environment. Appl. Environ. Microbiol. **69:**2884–2892.
6. **Blanquet, S., et al.** 2004. A dynamic artificial gastrointestinal system for studying the behavior of orally administered drug dosage forms under various physiological conditions. Pharm. Res. **21:**585–591.
7. **Buts, J. P., and P. Bernasconi.** 2005. *Saccharomyces boulardii*: basic science and clinical applications in gastroenterology. Gastroenterol. Clin. North Am. **34:**515–532.
8. **Castagliuolo, I., J. T. LaMont, S. T. Nikulasson, and C. Pothoulakis.** 1996. *Saccharomyces boulardii* protease inhibits *Clostridium difficile* toxin A effects in the rat ileum. Infect. Immun. **64:**5225–5232.
9. **Chiou, R. Y., R. D. Phillips, P. Zhao, M. P. Doyle, and L. R. Beuchat.** 2004. Ethanol-mediated variations in cellular fatty acid composition and protein profiles of two genotypically different strains of *Escherichia coli* O157:H7. Appl. Environ. Microbiol. **70:**2204–2210.
10. **Chong, Y., et al.** 2007. Human intestinal tissue tropism in *Escherichia coli* O157:H7—initial colonization of terminal ileum and Peyer's patches and minimal colonic adhesion *ex vivo*. Microbiology **153:**794–802.
11. **Czerucka, D., T. Piche, and P. Rampal.** 2007. Review article: yeast as probiotics—*Saccharomyces boulardii*. Aliment. Pharmacol. Ther. **26:**767–778.
12. **Dahan, S., et al.** 2003. *Saccharomyces boulardii* interferes with enterohemorrhagic *Escherichia coli*-induced signaling pathways in T84 cells. Infect. Immun. **71:**766–773.
13. **Desreumaux, P., et al.** 2010. *Saccharomyces cerevisiae* CNCM I-3856 reduces digestive discomfort and abdominal pain in subjects with irritable bowel syndrome: a randomized double-blinded placebo-controlled clinical trial, abstr. OP-181. Abstr. 18th United European Gastroenterology Week, Barcelona, Spain.
14. **FAO/WHO.** 2002. Report of a joint FAO/WHO working group on drafting guidelines for the evaluation of probiotics in food. Food and Agriculture Organization of the United Nations and World Health Organization, London, Ontario, Canada.
15. **Fitzhenry, R. J., et al.** 2002. Intimin type influences the site of human intestinal mucosal colonisation by enterohaemorrhagic *Escherichia coli* O157:H7. Gut **50:**180–185.
16. **Foligné, B., J. Dewulf, P. Vandekerckove, G. Pignède, and B. Pot.** 2010. Probiotic yeasts: anti-inflammatory potential of various nonpathogenic strains in experimental colitis in mice. World J. Gastroenterol. **16:**2134–2145.
17. **Foster, J. W.** 2004. *Escherichia coli* acid resistance: tales of an amateur acidophile. Nat. Rev. Microbiol. **2:**898–907.
18. **Ganzle, M. G., C. Hertel, J. M. Van der Vossen, and W. P. Hammes.** 1999. Effect of bacteriocin-producing lactobacilli on the survival of *Escherichia coli* and *Listeria* in a dynamic model of the stomach and the small intestine. Int. J. Food Microbiol. **48:**21–35.
19. **Gobert, A. P., et al.** 2007. Shiga toxin produced by enterohemorrhagic *Escherichia coli* inhibits PI3K/NF-kappaB signaling pathway in globotriaosylceramide-3-negative human intestinal epithelial cells. J. Immunol. **178:**8168–8174.
20. **Mahajan, A., et al.** 2009. An investigation of the expression and adhesin function of H7 flagella in the interaction of *Escherichia coli* O157:H7 with bovine intestinal epithelium. Cell. Microbiol. **11:**121–137.
21. **Marteau, P., M. Minekus, R. Havenaar, and J. H. Huis in't Veld.** 1997. Survival of lactic acid bacteria in a dynamic model of the stomach and small intestine: validation and the effects of bile. J. Dairy Sci. **80:**1031–1037.
22. **Minekus, M., P. Marteau, R. Havenaar, and J. H. Huis in't Veild.** 1995. A multicompartmental dynamic computer-controlled model simulating the stomach and small intestine. ATLA **23:**197–209.
23. **Ogawa, M., et al.** 2001. Inhibition of in vitro growth of Shiga toxin-producing *Escherichia coli* O157:H7 by probiotic *Lactobacillus* strains due to production of lactic acid. Int. J. Food Microbiol. **68:**135–140.
24. **Paton, J. C., and A. W. Paton.** 1998. Pathogenesis and diagnosis of Shiga toxin-producing *Escherichia coli* infections. Clin. Microbiol. Rev. **11:**450–479.
25. **Pradel, N., et al.** 2000. Prevalence and characterization of Shiga toxin-producing *Escherichia coli* isolated from cattle, food, and children during a one-year prospective study in France. J. Clin. Microbiol. **38:**1023–1031.
26. **Rangel, J. M., P. H. Sparling, C. Crowe, P. M. Griffin, and D. L. Swerdlow.** 2005. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982-2002. Emerg. Infect. Dis. **11:**603–609.
27. **Roering, A. M., et al.** 1999. Comparative survival of *Salmonella typhimurium* DT 104, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 in preservative-free apple cider and simulated gastric fluid. Int. J. Food Microbiol. **46:**263–269.
28. **Roxas, J. L., et al.** 2010. Enterohemorrhagic *E. coli* alters murine intestinal epithelial tight junction protein expression and barrier function in a Shiga toxin-independent manner. Lab. Invest. **90:**1152–1168.
29. **Takumi, K., R. de Jonge, and A. Havelaar.** 2000. Modelling inactivation of *Escherichia coli* by low pH: application to passage through the stomach of young and elderly people. J. Appl. Microbiol. **89:**935–943.
30. **Tamplin, M. L.** 2005. Inactivation of *Escherichia coli* O157:H7 in simulated human gastric fluid. Appl. Environ. Microbiol. **71:**320–325.
31. **Thanassi, D. G., L. W. Cheng, and H. Nikaido.** 1997. Active efflux of bile salts by *Escherichia coli*. J. Bacteriol. **179:**2512–2518.
32. **Tkalcic, S., et al.** 2003. Fecal shedding of enterohemorrhagic *Escherichia coli* in weaned calves following treatment with probiotic *Escherichia coli*. J. Food Prot. **66:**1184–1189.
33. **Wong, C. S., S. Jelacic, R. L. Habeeb, S. L. Watkins, and P. I. Tarr.** 2000. The risk of the hemolytic-uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 infections. N. Engl. J. Med. **342:**1930–1936.